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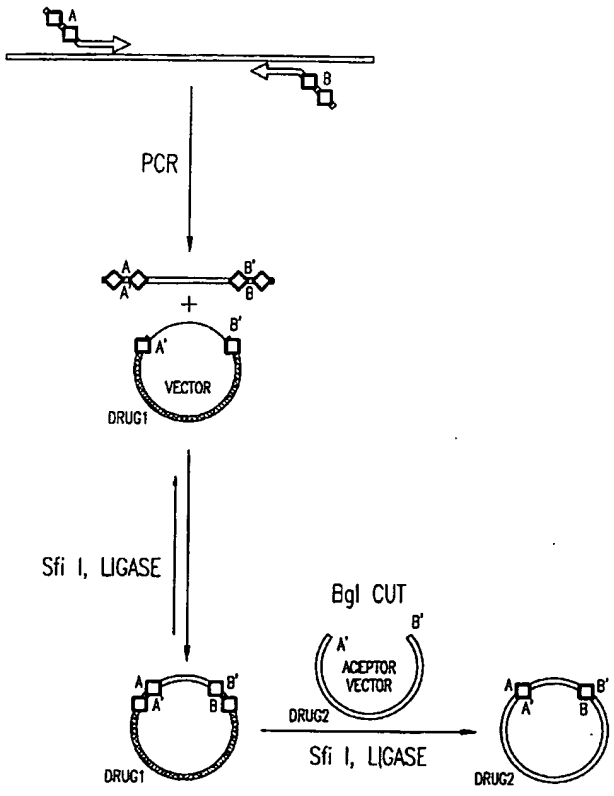
(54) Title: VECTORS FOR DIRECTIONAL CLONING



(57) Abstract: The invention provides vectors and methods for directional cloning.

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GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

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A. CLASSIFICATION OF SUBJECT MATTER
C12N15/10 C12N15/64 C12N15/66 C12N15/70 C12N9/22
G06F19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N G06F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, CAB Data, Sequence Search, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	AEBI M ET AL: "Sequence requirements for splicing of higher eukaryotic nuclear pre-mRNA." CELL. 21 NOV 1986, vol. 47, no. 4, 21 November 1986 (1986-11-21), pages 555-565, XP008052167 ISSN: 0092-8674	1-5, 14-18,26
Y	page 563, right-hand column, paragraph 2	1-3,14

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
11 November 2005	29. 11. 2005

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Hornig, H

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Form PCT/ISA/210 (second sheet) (January 2004)

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Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-26, 28-35, 36 partially, 40

A method for the directional subcloning of DNA fragments comprising: a) providing a first vector comprising a first selectable marker gene and a DNA sequence of interest, which DNA sequence of interest is flanked by at least two restriction enzyme sites (REs), wherein at least one of the flanking RE sites is a site for a first RE which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates complementary single-strand DNA overhangs, wherein at least one of the flanking RE sites is for a second RE which has infrequent restriction sites in cDNAs or ORFs from at least one species and generates ends that are not complementary to the overhangs generated by the first RE, wherein digestion of the first vector with the first RE and the second RE site generates a first linear DNA fragment which lacks the first selectable marker gene but comprises the DNA sequence of interest; b) providing a second vector comprising a second selectable marker gene which is distinguishable from the first selectable marker gene and non-essential DNA sequences, which non-essential sequences are flanked by at least two restriction enzymes sites, wherein at least one of the flanking RE sites in the second vector is for a third RE which generates complementary single-strand DNA overhangs that are complementary to the single-strand DNA overhang generated by the first restriction enzyme in the first linear DNA fragment, wherein at least one of the flanking RE sites in the second vector is for a fourth RE which generates ends that are not complementary to the ends generated by the first or third RE but can be ligated to the ends generated by the second RE, and wherein digestion of the second vector with the third RE and the fourth RE generates a second linear DNA fragment which lacks non-essential DNA sequences but comprises the second selectable marker, which second linear DNA fragment is flanked by ends which permit the oriented joining of the first linear DNA fragment to the second linear DNA fragment; and c) combining the first and second vectors, the first vector and the second linear DNA fragment, or the second vector and the first linear DNA fragment in a suitable buffer with one or more REs under conditions effective to result in digestion to yield a mixture comprising the first and second linear DNA molecules which are joined in an oriented manner. said method wherein at least one hapaxoterministic RE is used;

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2. claim: 27

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A method for producing a vector suitable for expression of an amino acid sequence of interest, comprising: combining at least two vectors in a suitable buffer with one or more restriction enzymes and optionally DNA ligase under conditions effective to result in digestion and optionally ligation to yield a mixture optionally comprising a third vector, wherein a first vector comprises a first selectable marker gene and a DNA sequence of interest, which DNA sequence of interest is flanked by at least two restriction enzyme sites, wherein two or more of the flanking restriction enzyme sites are sites for a first restriction enzyme which is a hapaxoterministic restriction enzyme, wherein digestion of the first vector with the first restriction enzyme generates a first linear DNA fragment which lacks the first selectable marker gene but comprises the DNA sequence of interest and a first pair non-self complementary single-strand DNA overhangs, wherein a second vector comprises a second selectable marker gene which is distinguishable from the first selectable marker gene and non-essential DNA sequences that optionally include a counterselectable gene, which non-essential DNA sequences are flanked by two or more restriction enzyme sites, wherein two or more of the flanking sites in the second vector are for a second restriction enzyme which is a hapaxoterministic restriction enzyme, wherein digestion of the second vector with the second restriction enzyme generates a second linear DNA fragment which lacks non-essential DNA sequences but comprises the second selectable marker gene and a second pair of non-self complementary single-strand DNA overhangs, wherein each of the second pair of the non-self-complementary DNA overhangs is complementary to only one of the single-strand DNA overhangs of the first pair of non-self complementary single-strand DNA overhangs and permits the oriented joining of the first linear DNA fragment to the second linear DNA fragment.

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3. claims: 37-38

A method of inducing expression of a DNA sequence of interest in a host cell, comprising contacting a recombinant host cell which is deficient in rhamnose catabolism, and has a recombinant DNA molecule comprising a rhamnose-inducible promoter operably linked to an open reading frame for a heterologous RNA polymerase, with rhamnose and an expression vector comprising a promoter for the heterologous RNA polymerase operably linked to a DNA sequence of interest.

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4. claim: 39

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A method comprising introducing a vector comprising a nucleic acid fragment encoding a barnase which lacks a secretory domain into a recombinant host cell which expresses barstar from a promoter which is constitutively expressed in procaryotic cells.

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5. claims: 41-43

A vector comprising an open reading frame 3' to a DNA fragment of no more than 30 base pairs, which DNA fragment comprise a ribosome binding site, a SgfI recognition site, and a sequence which, when present in mRNA enhances the binding of the mRNA to the small subunit of a eucaryotic ribosome; a vector comprising a SgfI recognition site, a sequence which comprises ATG and which sequence when present in mRNA, enhances the binding of the mRNA to the small subunit of a eucaryotic ribosome, and an open reading frame which begins at the ATG in the sequence;

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6. claims: 44-102, (116-119,121-126,128) partially

A vector comprising a SgfI recognition site 5' to a recognition site for a first restriction enzyme which generates blunt ends; a vector comprising a first open reading frame which includes a SgfI recognition site and a recognition site which is not in the open reading frame for a restriction enzyme that has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends; a vector comprising a ribosome binding site which optionally overlaps by one nucleotide with a SgfI recognition site and a recognition site which is not in the open reading frame for a restriction enzyme that has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends; a vector comprising a first open reading frame which includes a recognition site for a first restriction enzyme that generates a 3' TA overhang and a recognition site for a second restriction enzyme that is not in the open reading frame generates blunt ends; a support comprising a plurality of recombinant vectors; a process to prepare said support; a library of recombinant cells comprising said recombinant vectors;

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7. claims: 103-114, (116-119,121-126) partially

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A vector comprising a first open frame which includes a PmeI recognition site and is linked at the 5' end by a recognition site for a first restriction enzyme that generates complementary single-strand DNA overhangs; a support comprising a plurality of recombinant vectors, wherein at least one recombinant vector was prepared by using said vector including said PmeI site; a method to prepare said support; a library of recombinant cells comprising said recombinant vectors;

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8. claims: 115,120,127 (121-126,128) partially,129,130

A support comprising a plurality of recombinant vectors, two or more of which comprise an open reading frame for a different polypeptide, wherein at least one recombinant vector comprises a promoter and an open reading frame which is flanked by two exchange site; a method to prepare said support; a library of recombinant cells comprising recombinant vectors or a library of recombinant vectors comprise an open reading frame for a different polypeptide; a method to prepare a plurality of mutagenized recombinant vectors: comprising providing DNAs comprising a plurality of mutagenized open reading frames flanked by two restriction enzyme sites for a first restriction enzyme which is a hapaxoterministic restriction enzyme and generates a first pair of non-self complementary single-stranded DNA overhangs; a library of recombinant cells or a library of recombinant vectors, a plurality of which recombinant vectors comprise said mutagenized recombinant vectors;

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9. claims: 131-141

A method for performing genetic analysis, comprising a) populating a database of genetic data with a plurality of genetic records; b) querying the database of genetic data to identify a first subset of genetic records; wherein each record has at least one recognition site for one predetermined restriction enzyme or for a restriction enzymes included in a set of predetermined restriction enzymes; and c) determining a set of statistics associated with the restriction enzyme recognition sites for at least a second subset of genetic records in the first subset; A computerized system for genetic analysis, comprising a database of genetic data; a processor; a set of one or more programs executed by the processor causing the processor to: query the database of genetic data to identify a first subset of genetic records; wherein each record has at least one recognition site for one predetermined restriction enzyme or for restriction enzymes included in a set of predetermined restriction enzymes, and; determine a set of statistics associated with the restriction enzyme recognition sites for at least a second subset of genetic records in the first subset;

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Y	abstract	88-97, 99,100
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A	the whole document	1-36,40, 44-102
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A	the whole document	1-36,40, 44-102

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
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